Fractionation of Stable Isotopes of Sulfur by Microorganisms and Their Role in Deposition of Native Sulfur[1]

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Sulfate-reducing bacteria are believed to have played an important role in the formation of elemental sulfur in such deposits as those in the coastal region of Texas, Louisiana, and Mexico. Their suspected role was the production of sulfide from sulfate contained in the water of the deposit. The sulfide apparently became oxidized chemically to elemental sulfur (Hunt, 1915; Wolf, 1925; Thiel, 1930; Ginter, 1934; Murzaiev, 1937; and Taylor, 1938). The elemental sulfur occurs as crystal seams or wall deposits in the porous calcite cap rock of salt domes (Shearon and Pollard, 1950; Feely, 1956). The sulfur is located within 2500 feet of the surface in deposits ranging up to 300 feet in thickness. The columns of rock salt beneath the deposits are circular to elliptical in shape and extend downward 12,000 to 30,000 feet below the surface. The cap rock that covers the rock salt consists of a layer of anhydrite which grades upward into transition rock containing gypsum, calcite, and sulfur, and, above this, cavernous calcite rock. The sulfur is generally most abundant in the transition rock. The cap rock is buried beneath sedimentary deposits of gravel, sand, and limey shale. The pores and cavities of the cap rock contain brine. Petroleum, hydrogen sulfide, and sulfide waters are frequently found together with sulfur in the cap rock of the salt domes. It is significant that calcite and sulfur appear to have been formed at the same time and to have increased proportionately while the sulfate disappeared (Taylor, 1938).

The possibility that isotope data might provide evidence of the kinds of transformations of sulfur that occurred in the geologic deposits seemed likely from the fact that the lighter stable isotope, S32, is somewhat more reactive chemically than the heavier isotope, S34. Isotope fractionation is a natural phenomenon (Thode, 1949; Ingerson, 1953; Rankama, 1954) and, consequently, the sulfur product would be expected to contain somewhat more S32 and less S34 than the source substance. Therefore, the S32:S34 ratio of the sulfur product would be greater than that of the reactant.

There are differences in the relative amounts of the stable sulfur isotopes, S32 and S34, in natural materials. Although the S32:S34 ratio of the sulfur in any single kind of terrestrial substance such as seawater, sulfide ores and salt deposits, may show considerable variation, sulfates are generally richer in S34, whereas sulfides and sulfur of biologic origin are poorer in S34 and richer in S32 (Thode *et al.*, 1949; Trofimov, 1949; Szabo *et al.*, 1950; Macnamara and Thode, 1950, 1951; and Macnamara *et al.*, 1952). The range of the S32:S34 ratios of all kinds of natural sulfur materials varies as much as 7 per cent, as is to be expected from thermodynamic considerations (Tudge and Thode, 1950).

It was reported by Thode *et al.* (1951) that reduction of sulfate by a sulfate-reducing bacterium under laboratory conditions brought about some fractionation of the stable sulfur isotopes. This assumes particular significance because Thode *et al.* (1954) found that the sulfur in the gypsum and anhydrite of the geologic formation where sulfur was located had lower S32:S34 ratios than the sulfur of the sulfide or elemental sulfur occurring in the same formation. This suggests that if the sulfide and sulfur had been derived from the sulfate, the products had become depleted in S34 or enriched in S32. Tests made on 10 different salt domes of Texas and Louisiana showed that if they had been derived from the surrounding sulfate, the isotopes of the native sulfur had become fractionated from 2.4 to 5.6 per cent (avg 3.9 per cent)[3] and the sulfur of the associated sulfide 3.4 to 6.2 per cent (avg 4.6 per cent).[4] These values suggest that considerably greater fractionation had occurred than the 1.0 to 1.2 per cent, noted by Thode *et al.* (1951), effected by the sulfate-reducing bacterium.

Butlin (1953) concluded that the elemental sulfur that accumulated in certain Cyrenaican lakes of North Africa was derived from sulfate by bacterial action. This sulfur was found by Macnamara and Thode (1951)

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[3] The percentage fractionation for the native sulfur

$$= \frac{(\text{S32:S34 for S}^0) - (\text{S32:S34 for SO}_4^{=})}{\text{S32:S34 for SO}_4^{=}} \times 100$$

[4] The percentage fractionation for the sulfide-sulphur

$$= \frac{(\text{S32:S34 for S}^{=}) - (\text{S32:S34 for SO}_4^{=})}{\text{S32:S34 for SO}_4^{=}} \times 100$$

to have become fractionated 3.2 per cent compared to the sulfur of the sulfate from which it was presumed to have been derived.

Although there would be fractionation of the sulfur during reduction of sulfate to sulfide by both chemical and biologic action, it is unlikely that the reaction was nonbiologic because the temperature during sulfur deposition was too low for chemical reduction of sulfate. Ginter (1934) concluded that there is no factual evidence that sulfate was reduced to sulfide by organic materials through strictly chemical reactions "at the reasonably low temperatures of the earth's sedimentary rocks." Further evidence for this conclusion was reported by Jones *et al.* (1956).

The claim that sulfate-reducing bacteria were implicated in formation of the sulfur is supported by the recovery of the bacteria from drill core material consisting of rock fragments often containing sulfur (ZoBell, 1946; Miller, 1949), and the fact that environmental conditions in the deposits were favorable for development of the bacteria. The junior author also recovered sulfate-reducing bacteria in 1948 from formation waters of sulfur mines issuing at the ground surface. Nevertheless, mere recovery of the bacteria from core materials and formation waters at the surface is more suggestive than proof of their occurrence and active development in the formation because they may have become introduced by the drilling mud or may have developed on the well casing (Miller, 1949; Davis and Updegraff, 1954).

The following investigation was undertaken to obtain further information on the ability of the sulfate-reducing bacterium, *Desulfovibrio desulfuricans*, to fractionate the stable isotopes of sulfur. The ability of the sulfur-oxidizing bacterium, *Thiobacillus thiooxidans*, to fractionate the sulfur isotopes during oxidation of elemental sulfur was also determined.

Materials and Methods

In the initial experiments, the sulfate-reducing bacteria were cultivated in media in glass-stoppered bottles (Starkey, 1938), and analyses of one of the numerous replicate culture solutions were made at each incubation period. In subsequent experiments, a 5-L mechanically agitated fermentor (Bartholomew *et al.*, 1950) was used, by means of which it was possible to recover, periodically, sulfide produced by sulfate reduction, to withdraw samples of the culture medium, and to make additions of substrate or neutralizing solution.

A diagram of the fermentor and associated equipment is shown in figure 1.

The bacterium was cultivated in continuously stirred solution medium in the fermentor (*7*) held in a constant temperature water bath (*6*). The stirrer, which operated at approximately 500 rpm, dispersed undissolved material and prevented development of local areas of low sulfate concentration. A stream of sterile nitrogen gas entered the solution through (*A*). The gas was freed of oxygen by passage through a solution of alkaline pyrogallol in the gas-washing bottle (*1*) and over glowing metallic copper held in a tube in a furnace (*2*). Absence of oxygen was checked by an alkaline solution of reduced methylene blue in a tube (*3*), through which the gas was passed. Approximately every 2 days the copper was regenerated by clamping off the system and passing hydrogen through the tube for 30 to 60 min. The gas stream was passed through the drying tube of dehydrite (*4*) and the tube of sterile cotton (*5*) which assured sterility of the gas. Additional substrate was added to the medium at (*B*) through a funnel (*8*). With positive gas pressure, samples of medium were withdrawn at (*C*) through funnel (*9*). Sulfide picked up by the gas stream left the fermentor at (*D*) and was absorbed by solutions of lead nitrate in the gas-washing bottle (*10*) and tubes (*11*).

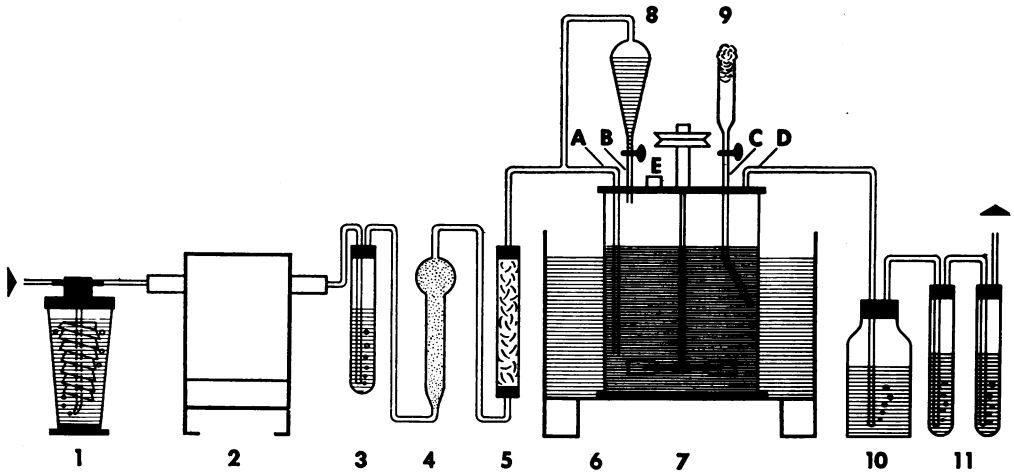

FIG. 1. Fermentor system. Nitrogen gas passed through alkaline pyrogallol at *1*, over metallic copper in electric furnace *2*, through reduced methylene blue *3*, drying reagent *4*, sterile cotton *5*, and to the fermentor *7*, and thence through $Pb(NO_3)_2$ at *10* and *11*. The fermentor was held in a constant temperature water bath *6*. Substrate was added by separatory funnel *8*; medium was sampled through sample funnel *9*.

The solutions of lead nitrate were replaced as needed. To recover the lead sulfide the liquid was centrifuged, the solids were washed 4 to 5 times with distilled water, and the supernatant liquid was passed through tared filter paper at each step. The materials were dried at 50 to 100 C. Only the principal portion of lead sulfide, that which had not been collected on the paper, was used for isotope analysis.

When insoluble material, such as calcium sulfate, was used in the medium, this was sterilized separately and added aseptically through the port (*E*). The media were similar to that of Starkey (1938) in which sodium lactate was the only organic constituent. Various concentrations of sodium lactate, magnesium sulfate, and calcium sulfate were used. The sulfide content of the culture medium was determined by iodine titration. Sulfate was determined gravimetrically as the barium salt. Isotope determinations of the S32:S34 ratios of the sulfate used to prepare the media and of the sulfide produced from sulfate by the bacteria were made by means of a dual collector mass spectrometer.[5]

Results

In preliminary experiments, the sulfate-reducing bacterium was cultivated in solution medium containing from 0.3 to 5.0 per cent $MgSO_4 \cdot 7H_2O$. The 500-ml glass-stoppered bottles were completely filled with the medium. The incubation temperature was 28 C. High concentrations of sulfate were used so that the amount of sulfate-sulfur reduced to sulfide would be only a small portion of the total quantity. The factor that limited the amount of sulfide produced was the amount of sodium lactate in the medium. In media with 0.3 and 5.0 per cent $MgSO_4 \cdot 7H_2O$, 13 and 5 per cent, respectively, of the sulfate-sulfur was reduced to sulfide. Development of the bacterium in the medium with the low sulfate concentration resulted in no detectable fractionation, but tests on two culture solutions containing the larger amount of sulfate showed 1.4 and 1.5 per cent fractionation of the sulfur isotopes.

The bacterium was cultivated in the fermentor in additional experiments designed to determine the conditions affecting the degree of fractionation. Only two representative experiments of seven conducted will be discussed in detail.

Four liters of the following medium were used in the fermentor:

Tap water	1000 ml
Na-lactate	18.0 g
$CaSO_4 \cdot 2H_2O$	21.0 g
NH_4Cl	2.0 g
$K_2HPO_4 \cdot 3H_2O$	1.0 g
$CaCO_3$	1.2 g
Mohr's salt	0.2 g

[5] Isotope determinations were made by J. L. Kulp and H. W. Feely. For details of analysis, see Feely, 1956.

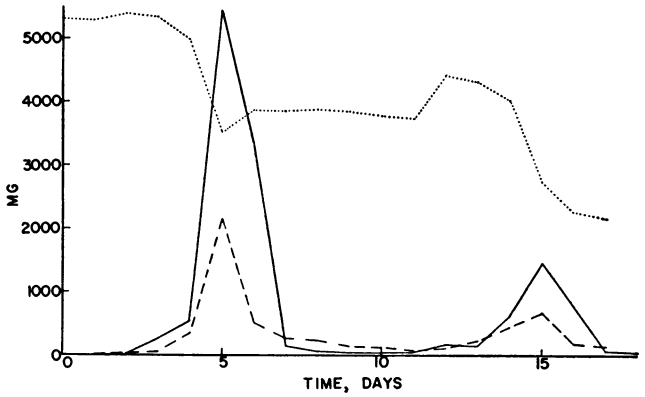

Fig. 2. Sulfate reduction in medium containing calcium sulfate as the source of sulfate and incubated at 28 C. Soluble sulfate-sulfur (·····); sulfide-sulfur in culture substrate (------); sulfide-sulfur recovered from the gas stream (———).

The reaction was adjusted to pH 7.1. The temperature was kept at 28 C. The inoculum consisted of 8 ml of a 7-day culture of *D. desulfuricans*.

The pH increased to 7.9 before the bacterium made appreciable growth and remained between 7.8 and 8.4 for most of the incubation period of 18 days. Sixty hours elapsed before growth was evident. As shown by figure 2, reduction of sulfate increased rapidly after initiation of growth. The maximum amount of sulfide was produced on the fifth day after which there was an abrupt decrease, and little or no sulfide was produced between the seventh and thirteenth days. The increase in activity toward the end of the experiment was due to addition of 22.9 g of sodium lactate on the eleventh day.

Practically 100 per cent of the sulfur provided as sulfate was accounted for as sulfide or residual sulfate. Calculations of the amount of sodium lactate required to produce the sulfide that was recovered according to the reaction of Baars (1930) indicated that all of the organic material had been broken down and that the amount of lactate was the limiting factor for growth.

The isotope analyses made on 16 samples of sulfide obtained at different periods of development of the culture are shown in table 1 and figure 3. The S32:S34 ratio for all the sulfate used in these experiments was 22.16 ± 0.02. The rate of sulfate reduction affected the degree of fractionation of the sulfur isotopes. Generally, the S32:S34 ratios were greater when the growth rate was slow.

In an additional experiment an attempt was made to decrease the rate of growth by keeping the amount of the energy source low by adding it in small increments throughout the incubation period. In another experiment the lactate concentration was similarly regulated, and in addition, the temperature was held at 20 C. There was somewhat more rapid sulfate reduction than expected and, as shown by figures 4 and 5, the rates were uneven. Nevertheless, in both cases there was

TABLE 1. *Sulfide production and fractionation of stable isotopes of sulfur by Desulfovibrio desulfuricans cultivated at 28 C*

Sample No.	Incubation Period	Sulfide S in PbS	Sulfate Reduced†	No. of Isotope Determinations	S32:S34	Fractionation
	*hr**	*mg*	%			%
1	44	996	6.3	4	22.28 ± 0.01	0.54 ± 0.14
2	8	2168	20.0	4	22.27 ± 0.01	0.50 ± 0.14
3	4	1931	32.2	2	22.23 ± 0.01	0.32 ± 0.14
4	5	1448	41.4	2	22.23 ± 0 01	0.32 ± 0.14
5	4	1394	50.2	2	22.28 ± 0.01	0.54 ± 0.14
6	3	1248	58.1	2	22 28 ± 0.01	0.54 ± 0.14
7	9	317	60.1	2	22.31 ± 0.01	0.68 ± 0.14
8	14	191	61.3	2	22 36 ± 0.01	0.90 ± 0.14
9	41	103	62.0	2	22.38 ± 0.01	0.99 ± 0.14
10	68	115	62.7	2	22.45 ± 0.01	1.31 ± 0.14
11	59.5	387	65.2	2	22.32 ± 0.01	0.72 ± 0.14
12	25.5	901	70.9	2	22.23 ± 0.01	0.32 ± 0.14
13	7	615	74.8	2	22.17 ± 0.01	0.05 ± 0.14
14	6	474	77.8	2	22.14 ± 0.01	−0.09 ± 0.14
15	24	856	83.2	2	22.15 ± 0.01	−0.05 ± 0.14
16	43	106	83.9	2	22.27 ± 0.01	0.50 ± 0.14
Totals.....	365	13,250				

* Periods are calculated from the time that sulfide first appeared in the culture substrate. This was 60 hr after the medium was inoculated.

† The initial sulfate-S in the substrate was 3943 ppm.

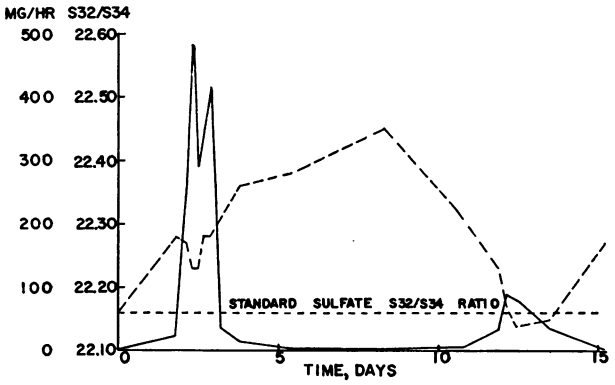

FIG. 3. Relation between rate of sulfate reduction and fractionation of sulfur isotopes in a medium containing calcium sulfate as the source of sulfate and incubated at 28 C. Rate of production of sulfide-sulfur, mg per hr (———); S32:S34 ratio of the sulfide-sulfur recovered from the gas stream (------).

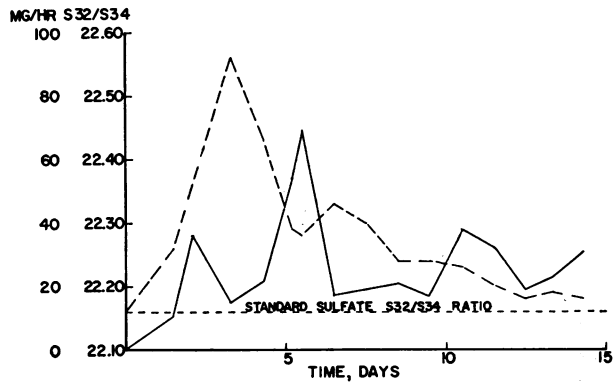

FIG. 4. Relation between rate of sulfate reduction and fractionation of sulfur isotopes in a medium containing calcium sulfate as the source of sulfate, with limited amount of Na-lactate, and incubated at 28 C. Rate of production of sulfide-sulfur, mg per hr (———); S32:S34 ratio of sulfide-sulfur recovered from the gas stream (------).

generally greater fractionation during periods of slow sulfate reduction, increase in fractionation with decrease in reduction rate, and decrease in fractionation with increase in reduction.

In another experiment, conditions were more favorable for a slow and fairly steady rate of sulfate reduction, and this resulted in a high degree of fractionation of the stable sulfur isotopes. The concentration of soluble sulfate was kept high by substituting 6 per cent $MgSO_4 \cdot 7H_2O$ for the relatively insoluble calcium sulfate used in other experiments. No difficulty was encountered in cultivating the bacterium in this medium. Likewise, Miller (1949) noted that sulfate-reducing bacteria of both marine and nonmarine origin were tolerant to relatively high salt concentrations. Sodium lactate was added in small increments during the course of the experiment, making a total of 17.1 g for the 4 L of medium. To avoid a long lag period the medium was inoculated with a large number of cells. The inoculum consisted of washed cells having a packed volume of 0.4 ml. They were harvested from 500 ml of a culture medium that contained 3 per cent $MgSO_4 \cdot 7H_2O$ and 1.8 per cent sodium lactate and that was incubated for 8 days at 19 C. The fermentor was held at from 8.9 to 17.8 C. The average temperature for the first 30 days was 16 C and for the last 15 days was 9.6 C.

There was evidence of growth after only 18 hr. As shown in figure 6, sulfide production increased gradually until the twenty-seventh day after which it remained

fairly constant. This was the slowest growth rate of all the experiments. This prolonged period of very slow sulfide production resulted in marked fractionation. As shown in table 2 and figure 7, the percentage fractionation varied from 2.0 to 2.7 with an average of 2.4. The relatively high fractionation of the sulfur is believed to have been due to the very slow rate of reduction of sulfate to sulfide and to the high concentration of soluble sulfate, only a small portion of which was reduced.

Although the amount of sodium lactate was kept low it was always sufficient to meet the requirements of the bacterium. The pH of the substrate increased slowly from an initial value of 7.2 to a final value of 8.5.

A portion of the residual sulfate in the fermentor at the end of the 45-day incubation period had a S32:S34 ratio of 22.12 ± 0.02. This was just significantly lower than 22.16 ± 0.02, the S32:S34 ratio of the sulfate from which it was produced. Decrease in the ratio would result from preferential use of S32 during sulfate

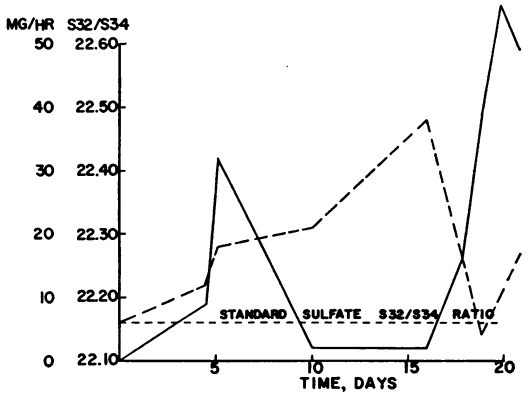

FIG. 5. Relation between rate of sulfate reduction and fractionation of sulfur isotopes in a medium containing calcium sulfate as the source of sulfate, with limited amount of Na-lactate, and incubated at 20 C. Rate of production of sulfide-sulfur, mg per hr (———); S32:S34 ratio of sulfide-sulfur recovered from the gas stream (------).

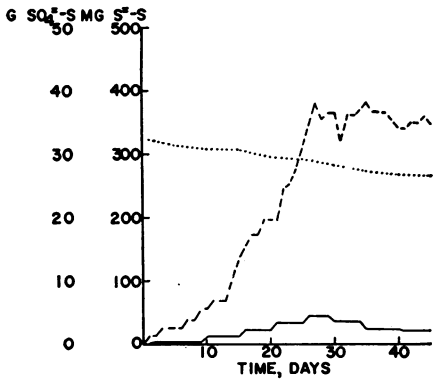

FIG. 6. Sulfate reduction, in medium containing magnesium sulfate as the source of sulfate and incubated at low temperatures. Soluble sulfate-sulfur (·····); sulfide-sulfur in culture substrate (------); sulfide-sulfur recovered from gas stream (———).

TABLE 2. *Sulfide production and fractionation of stable isotopes of sulfur by Desulfovibrio desulfuricans cultivated at low temperatures*

Sample No.	Incubation Period	Sulfide S in PbS	Sulfate Reduced	S32:S34	Fractionation
	*hr**	*mg*	*%*		*%*
17	200	21.2	4.4	22.66 ± 0.02	2.26 ± 0.18
18	142	65.8	4.5	22.75 ± 0.02	2.66 ± 0.18
19	120	112.7	8.3	22.75 ± 0.01	2.66 ± 0.14
20	120	167.8	10.0	22.71 ± 0.02	2.48 ± 0.18
21	96	174.6	13.1	22.71 ± 0.01	2.48 ± 0.14
22	120	180.8	16.0	22.68 ± 0.01	2.35 ± 0.14
23	144	134.5	16.8	22.65 ± 0.01	2.21 ± 0.14
24	120	102.0	18.4	22.60 ± 0.01	1.99 ± 0.14
Totals...	1062	959.4			

* Periods are calculated from the time that sulfide first appeared in the culture substrate; this was 18 hr after the medium was inoculated.

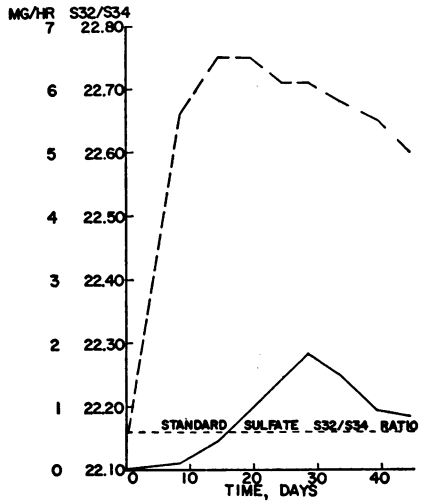

FIG. 7. Relation between rate of sulfate reduction and fractionation of sulfur isotopes in a medium containing magnesium sulfate as the source of sulfate and incubated at low temperatures. Rate of production of sulfide-sulfur, mg per hr (———); S32:S34 ratio of sulfide-sulfur recovered from the gas stream (------).

reduction. The fact that the decrease was slight compared to the increase in the ratio of the sulfide produced from the sulfate is to be expected, for only a very small portion of the sulfate was reduced.

Whereas the maximum fractionation noted in these experiments was 2.7 per cent, it was improbable that this is the maximum fractionation that can be effected by the bacterium. It seems likely that with slower metabolic activity, there would be still greater fractionation.

Tests for fractionation by Thiobacillus thiooxidans. If organisms other than sulfate-reducing bacteria fractionate the sulfur isotopes, differences in the S32:S34 ratios of the sulfur of natural materials may be attributed to various biologic effects. Changes in iso-

tope ratios might be the result of multiple reactions complicating their interpretation. Isotope analysis of elemental sulfur and of sulfate formed as the result of aerobic oxidation of elemental sulfur by the autotrophic sulfur bacterium, *T. thiooxidans*, showed no fractionation under varying conditions where from 2.6 to 67.1 per cent of the elemental sulfur in the medium had become oxidized. This does not eliminate the possibility that there is fractionation during its oxidation of soluble sulfur substrates, but this was not tested.

Discussion

In the bacterial reduction of sulfate certain factors may be concerned that would not affect strictly chemical transformations. Sulfate probably moves into the cell where it is reduced and sulfide probably moves out. Furthermore, absorbed sulfate may be released to the culture solution. Isotope fractionation would be affected by differences in the rates of: (1) penetration into the cell by the two isotopes, (2) reduction of the isotopes in the cell, and (3) excretion of the isotopes from the cell. If both isotopes enter the cell at the same rate and fail to be released as sulfate once they are absorbed, it is probable that there would be little fractionation. If, on the other hand, they could pass out of the cell readily after having entered, and S34 was more slowly reduced than S32, conditions would be favorable for fractionation. There would be an increased fractionation if the heavier isotope moved into the cell less rapidly than the lighter one and also was reduced more slowly.

Appreciable fractionation requires a high concentration of sulfate about the cell at all times to insure that the medium does not become diluted in the preferentially utilized isotope. In case of deficiency of sulfate, there would be a tendency for all sulfate to be reduced irrespective of its atomic weight. Therefore, fractionation would be favored by an initial high concentration of soluble sulfate, by microbial transformation of only a small portion of the available sulfate, by continuous mixing of the medium, and by a slow rate of sulfate reduction. It was noted repeatedly that, when a large portion of the soluble sulfate had become reduced, there was a decrease in fractionation even though the rate of growth remained fairly constant.

When used at relatively high concentrations, the soluble sulfate salt, $MgSO_4 \cdot 7H_2O$, should be more favorable for isotope fractionation than the more insoluble $CaSO_4 \cdot 2H_2O$. The latter was used to simulate conditions in the natural formations where sulfur is deposited. During periods of rapid sulfate reduction in the medium with calcium sulfate, the solution failed to remain saturated with sulfate, and selective utilization of S32 was confined to the portion of sulfate in solution. Furthermore, equilibrium between the isotopes in the insoluble and soluble sulfate was not maintained.

There was significant fractionation with 54 of 62 samples of sulfide produced by the sulfate-reducing bacterium cultivated in the fermentor, and none of the determinations showed significant increase in the amount of S34 relative to S32. A general trend for high fractionation was evident during periods of slow sulfide production. This is indicated by the fact that the percentage fractionation averaged 0.94 for 43 samples obtained during periods when the rate of sulfide production was below 40 mg per hr, whereas at periods of more rapid sulfide production the average was 0.33 for 19 samples. In the experiments where 6 per cent $MgSO_4 \cdot 7H_2O$ was used as the sulfate source, the percentage fractionation averaged 2.02 when the rate of sulfide production was 8 mg per hr or less.

It is not possible to establish a satisfactory correlation between rates of sulfide production and percentage fractionation because all of the sulfide was not removed from the culture solution by the gas stream at each period. The sulfide produced at one period and not removed by the gas stream would become mixed with that produced at subsequent periods and this would obscure the actual degree of fractionation at the latter periods.

In calculating the percentage fractionation, the isotope ratios of the source sulfate, $CaSO_4 \cdot 2H_2O$ or $MgSO_4 \cdot 7H_2O$, have been used as the base, and the differences between these values and those of the sulfide derived from the sulfate have been determined. The S32:S34 ratio of both salts was 22.16 ± 0.02. However, the percentage fractionation reported by others was based on differences of the S32:S34 ratios of the sulfide or sulfur found in nature and those of the residual sulfate (Rankama, 1954; Thode *et al.*, 1954). The maximum concentration of S32 was found in sample 18 where the S32:S34 ratio of the sulfide-sulfur was 22.75 and that of the residual sulfate was 22.12. Thus, the percentage fractionation from these values is 2.85 whereas it is 2.66 when using the ratio of the initial sulfate-sulfur as the base.

When calculating fractionation of sulfur isotopes by sulfate-reducing bacteria in nature it has been assumed that the sulfate source, whether it be sea water or the sulfate in the salt domes, is an "infinite pool" and changes little in isotope ratio. Sulfate in the brines and in the gypsum bathed by the brines in the formations where sulfur is deposited may be depleted gradually in S32 as hydrogen sulfide is formed, and some of the observed differences between the S32:S34 ratios of sulfate and sulfide may be due to this depletion of the dissolved sulfate in S32.

The sulfate-sulfur of a Cyrenaican lake of North Africa had an S32:S34 ratio similar to that of sea water,

indicating that there was a large reservoir of sulfate that had not been greatly enriched with S34 (Macnamara and Thode, 1951). A fractionation of 3.2 per cent was recorded between the sulfate and elemental sulfur of this lake, which is only slightly higher than the maximum fractionation of 2.7 per cent noted for the reduction of sulfate to sulfide in the experiments reported herein. In the salt domes, however, the residual sulfate associated with the elemental sulfur shows evidence of having become depleted in S32, the degree of depletion differing with the localities (Thode *et al.*, 1954). The S32:S34 ratio of the sulfate of the salt dome at Lake Washington, Plaquenimes Parish, Louisiana, was 21.16, whereas the average ratio for sulfate of 9 other sulfur wells was 21.36. This suggests that there had been considerable depletion in S32.

According to results of Thode *et al.* (1954) the fractionation that had taken place in the formation of sulfide in 10 salt domes of Texas and Louisiana varied from 3.4 to 6.2 per cent. Very recent results of Feely on materials of three salt domes of Texas showed S32: S34 ratios of sulfide-sulfur that were from 1.0 to 3.2 per cent higher than those of the sulfate in the salt and anhydrite rock. These latter values are within the range that is to be expected for sulfide produced through bacterial sulfate reduction from the results herein reported.

Since all salt domes which harbor commercial quantities of sulfur contain hydrocarbons (Taylor, 1938), the fact that some suggestive evidence was obtained indicating a capability of sulfate-reducing bacteria to oxidize certain constituents of crude oil (Jones, 1955) was considered important. Kuznetsov (1950) stated that sulfate reduction at the expense of organic matter in petroleum is extremely slow and depends on the composition of the petroleum. Updegraff and Wren (1954) agreed that the process is very slow, if indeed it occurs at all. The possibility that sulfate-reducing bacteria are supported by hydrocarbons or other compounds contained in connate water associated with the formations has not been determined.

Acknowledgments

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Summary

The degree of fractionation of the stable sulfur isotopes, S32 and S34, resulting from reduction of sulfate to sulfide by *Desulfovibrio desulfuricans* and oxidation of elemental sulfur to sulfate by *Thiobacillus thiooxidans* was determined.

There was no fractionation during the reduction of sulfate to sulfide when the sulfate-reducing bacterium was cultivated in stationary medium containing 0.3 per cent $MgSO_4 \cdot 7H_2O$, but there was 1.4 to 1.5 per cent fractionation in stationary medium containing 5.0 per cent of the salt. In continuously agitated media in a fermentor, the amount of fractionation of the sulfur isotopes varied from negligible values to 2.7 per cent, depending on the rate of sulfate reduction and the concentration of sulfate. Fractionation was greatest where the rate of sulfate reduction was exceedingly slow and the amount of sulfate in solution was high, and when only a small portion of the sulfate became reduced and the medium was kept uniform by continuous stirring. There was less fractionation in media with calcium sulfate than with magnesium sulfate. Low temperature was an effective means of keeping the metabolic rate slow. During incubation at low temperatures the lag period was shortened by inoculation with a large number of cells.

The maximum fractionation of the sulfur isotopes brought about by the sulfate-reducing bacterium was within the range of the apparent fractionation that occurred during deposition of elemental sulfur in the cap rock of the sulfur deposits of the salt domes of Texas and Louisiana. This supports the hypothesis that sulfate-reducing bacteria were responsible for the first step in the formation of the sulfur in the deposits, that of production of hydrogen sulfide from sulfate in solution.

No fractionation of the sulfur isotopes was noted during oxidation of elemental sulfur to sulfate by *Thiobacillus thiooxidans.*

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